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Use of Microdosing and Accelerator Mass Spectrometry to Evaluate the Pharmacokinetic Linearity of a Novel Tricyclic GyrB/ParE Inhibitor in Rats

M. A Malfatti, V. Lao, C. L. Ramos, V. S. Ong, K. W. Turteltaub

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USE OF MICRODOSING AND ACCELERATOR MASS SPECTROMETRY TO EVALUATE THE PHARMACOKINETIC LINEARITY OF A NOVEL TRICYCLIC GyrB/ParE (TriBE) INHIBITOR IN RATS

Michael A. Malfatti[1#], Victoria Lao[1], Courtney L. Ramos[2], Voon, S. Ong[3], Kenneth W. Turteltaub[1]

[1]Biosciences and Biotechnology Division, Physical and Life Sciences Directorate
Lawrence Livermore National Laboratory, Livermore, CA
[2]*Previous affiliation*: Cubist, San Diego, CA; *Current affiliation*: Touro University Nevada, College of Osteophathic Medicine, Henderson, NV
[3]Cubist, San Diego, CA

Running title: Microdosing to evaluate PK of a novel antimicrobial

#Corresponding author:
Michael A. Malfatti, PhD
Biosciences and Biotechnology Division
Lawrence Livermore National Laboratory
7000 East Avenue, L-452
Livermore, CA 94550
Phone: 925-422-5732
Email: malfatti1@llnlgov

ABSTRACT

Determining the pharmacokinetics (PK) of drug candidates is essential for understanding their biological fate. The ability to obtain human PK information early in the drug development process can help decide if future development is warranted. Microdosing was developed to assess human PK, at ultra-low doses, early in the drug development process. Microdosing has also been used in animals to confirm PK linearity across sub-pharmacologic and pharmacologic dose ranges. The current study assessed the PK of a novel antimicrobial preclinical drug candidate (GP-4) in rats as a step towards human microdosing studies. Dose proportionality was determined at 3 proposed therapeutic doses (3, 10, & 30 mg/kg) and PK linearity was assessed between a microdose and a pharmacologic dose in Sprague-Dawley rats. Plasma PK over the 3 pharmacologic doses was proportional. Over the 10-fold dose range the C_{max} and AUC had a 9.5 and 15.8 fold increase, respectively. PK from rats dosed with a ^{14}C-microdose vs. a ^{14}C-pharmacologic dose displayed dose linearity. The terminal $T_{1/2}$ was 5.1 h and 13.2 h, and the volume of distribution was 28.5 L/kg and 30.2 L/kg in the micro and therapeutic dosed animals, respectively. When normalized to 1.0 mg/kg, the AUC values were 244.9 ng·hr/mL for the microdose and 451.7 ng·hr/mL for the pharmacologic dose. This 1.8-fold difference in AUC following a 300-fold difference in dose is considered linear across the dose range. Based on the results, the PK from the microdosed animals was considered to be predictive of the PK from the therapeutically dosed animals.

INTRODUCTION

Developing new drugs is a long and costly process often taking more than 10 years and over $1 billion to get one drug to market (1). The huge cost of new drugs is a consequence of the high failure rate of potential candidates prior to approval. One of the contributing factors to the high attrition rate during development is poor pharmacokinetics (PK). Poor PK parameters are responsible for up to 40% of drug candidates failing to make it past the first in human studies (2-3). Being able to predict complete biodistribution profiles that include PK, tissue distribution, and route of elimination early in the drug development process would provide critical data for decisions about the continued development of a drug lead. These data are also useful for optimizing dosage regimes, potentiation of therapeutic efficacy, tailoring drug delivery systems and for evaluating safety. Early evaluation of these factors in humans would determine if further development is warranted before the initiation of costlier clinical trials.

Microdosing is a technique used to assess a compound's *in vivo* biological fate through the administration of sub-pharmacological doses of a ^{14}C-labeled drug candidate that produce virtually no adverse effects. Microdosing is used to down-select among multiple new chemical entities, to obtain preliminary human pharmacokinetic information for investigational new drugs early in the development process, and to confirm *in vitro* or *in silico* metabolic pathways *in vivo* (4-7). It should be noted that microdosing provides no data on efficacy or safety for the drug candidate. Microdosing studies have the potential to reduce the attrition rate of drugs because inadequate PK properties can be identified earlier in the development process saving time and money (8).

The linearity of the PK across the range of microdose to therapeutic dose is one of the fundamental concerns with microdosing studies. Although not comprehensive, there is a growing body of evidence showing that the PK parameters of several drugs are linear over a 50-1000 fold dose range (9, 8). In a seminal human study, Lappin et al. showed that the pharmacokinetics of 3 prescription drugs were essentially linear (within a factor of two) across a range of micro- and therapeutic doses. (10). Sandhu et al. used microdosing to examine

whether a pre-clinical drug candidate (7-deaza-2'-C-methyl-adenosine) displayed linear kinetics across a sub-pharmacological and a pharmacological dose range in an animal model before the initiation of human microdose study. The results showed that the PK properties were linear over a 50-fold dose range. (11)

The success of microdosing is dependent on the availability of ultrasensitive analytical methods that are able to measure drug concentrations in the low picogram to femtogram range (6). Accelerator mass spectrometry (AMS) is one of only a few current techniques ideally suited for this application due to its extreme sensitivity and selectivity. AMS is a technique for counting rare, long-lived isotopes independent of radioactive decay by measuring the mass ratio of the radioisotope of interest relative to a stable isotope of the element (12). AMS can be used to trace the fate of any molecule in *in vitro* systems or whole organisms if it is labeled with an isotope appropriate for AMS analysis. Because most biological materials contain carbon, the majority of AMS studies use ^{14}C as the radiotracer. AMS can detect and quantify a ^{14}C-labeled compound in a biological matrix with 1%-3% precision at levels ranging from approximately 10 pmol ^{14}C to 1 attomol (1×10^{-18}) ^{14}C in samples containing as little as 250 μg of total carbon (12-14). More recently, methods have been developed that allow even greater sensitivity using samples as small as a few micrograms (15-16).

The sensitivity of AMS measurement gives this technique a number of major advantages over other methods for the detection of isotopes. Importantly, because of the extreme sensitivity, PK studies have the ability to determine kinetics and metabolism using ultra-low doses of chemical and radioactivity so as not to perturb the natural biological state. In addition, detailed pharmacokinetic data require frequent sampling, which is made possible with AMS detection, by virtue of the small sample sizes needed for analysis. AMS has been the method of choice for microdosing studies because the dose required for determining PK parameters is so low, there is little or no concern for drug efficacy or toxic affects.

The objective of the current study was to determine whether the pharmacokinetics of a novel GyrB/ParE inhibitor preclinical drug candidate (GP-4) displayed linear kinetics across a microdose and pharmacological dose range in rats.

This study served as a first step towards initiating a human microdose study, and as a preliminary investigation of the feasibility of using the rat as a model. The GyrB/ParE inhibitors are a novel class of antibacterial agents, the pyrimidoindoles, which inhibit two bacterial targets: the ATP-binding domains of bacterial DNA gyrase (GyrB) and topoisomerase IV (ParE). The pyrimidoindoles were designed to be highly potent against both enzymes, with broad-spectrum antibacterial activity and excellent selectivity versus other mammalian enzymes that utilize ATP (17).

The PK parameters for GP-4 were assessed over a 300-fold dose range by administering rats a microdose or pharmacological dose of ^{14}C-GP-4. Following drug administration blood was collected at specified time points up to 24 hours. GP-4 concentration in plasma was determined by quantifying the amount of ^{14}C radiolabel in the samples by AMS or LC/MS/MS. The PK parameters from both the microdose and pharmacologic dose were determined and compared. The results showed that the PK properties of GP-4 for the animals given a microdose were similar to those of the animals given a pharmacologic dose indicating linearity across the 300-fold dose range. Moreover, these results demonstrate the utility of AMS in providing exceptional sensitivity for conducting microdose studies.

MATERIALS AND METHODS

Chemicals and reagents. ^{14}C-radiolabeled GP-4 (specific activity 50 mCi/mmol) and unlabeled GP-4 were provided by Cubist Pharmaceuticals (San Diego, CA). Chemical and radiopurity was assessed by HPLC and determined to be > 99% pure. Captisol® was obtained from Ligand Pharmaceuticals, Inc. (La Jolla, CA), verapamil and methanesulfonic acid was purchased from Sigma-Aldrich Inc. (St. Louis, MO). All other reagents were of analytical grade or better.

Animals. Animal experiments were conducted at the Lawrence Livermore National Laboratory (LLNL) AAALAC accredited animal care facility. The protocols for animal experiments were reviewed and approved by the LLNL Institutional Animal Care and Use Committee prior to the initiation of any study. Six-to-eight week old male Sprague-Dawley rats weighing 250-300 g, with a surgically implanted jugular vein catheter, were obtained from Harlan Laboratories

(Livermore, CA). Animals were housed individually in polystyrene cages containing hardwood bedding and kept on a 12 h light/dark cycle in a ventilated room maintained at 24°C. Animals were provided food (standard lab chow) and water *ad libitum*.

Dose proportionality. Rats (n=3) were administered a single intravenous bolus dose of 3, 10, or 30 mg/kg GP-4 formulated in 20% Captisol in 0.05 M methane sulfonic acid, pH 3.5. Following dose administration, whole blood samples (approximately 0.3 mL) were collected from each animal via the jugular vein at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 hrs post dose, placed into Microtainer® tubes coated with lithium heparin (Becton Dickinson, Franklin Lakes, NJ) and placed on ice. Plasma was separated from the whole blood by centrifugation (10,000 x g for 2 min) within 1 hour of collection, the volume recorded and stored at -20°C until analysis by LC/MS/MS.

LC/MS/MS analysis. Each plasma sample together with an internal standard (50 ng/ml verapamil in acetonitrile: water 1:1) was directly injected into a Shimadzu LC-20AD HPLC system (Shimadzu, Columbia, MA) coupled to a AB-SCIEX API 3200Q Otrap triple quadrupole mass spectrometer with a TurboIon spray interface (AB SCIEX, Framington, MA). Samples were chromatographed using a Gemini-NX 3 µm C18 column (50 x 3 mm) (Phenomenex, Torrance, CA) operating at a flow rate of 0.6 mL/min initially using a solvent of 85% A (Solvent A: 12.5 mM ammonium formate, pH 4; solvent B: 0.1% formic acid in acetonitrile) for 1 min. This was followed by a linear gradient up to 95% B at 3.2 min. and held at 95% B until 3.8 min., then re-equilibrated to 85% A over 1.2 min. Quantitation was by multiple reaction monitoring (transitions of m/z ratios 435 to 418 for GP-4 and 455 to 165 for verapamil).

Microdose vs. therapeutic dose pharmacokinetics. To evaluate the pharmacokinetics of GP-4 in rodents at a microdose and pharmacologic dose, rats (n=3) were administered a single IV dose of 0.01 mg/kg or 3.0 mg/kg ^{14}C-GP-4 (specific activity 353 µCi/mmol and 1.26 µCi/mmol, respectively) formulated in 20% Captisol in 0.05 M methanesulfonic acid, pH 3.5. Following dose

administration, whole blood samples (approximately 0.3 mL) were collected from each animal via the jugular vein at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 hrs post dose, placed into Microtainer® tubes coated with lithium heparin (Becton Dickinson, Franklin Lakes, NJ) and placed on ice. Plasma was separated from the whole blood by centrifugation (10,000 x g for 2 min) within 1 hour of collection, the volume recorded and stored at -20°C until analysis by AMS.

AMS analysis. Preparation of the samples for radiocarbon analysis by AMS requires conversion of the samples to graphite. This procedure has been described previously (18). All the samples and reagents were handled carefully to avoid radiocarbon cross-contamination, including using disposable materials for any item that might come into contact with the samples. A 30 μL aliquot of each plasma sample was pipetted into a 6 X 55 mm quartz tube using aerosol resistant tips. All samples were subsequently dried under vacuum centrifugation. The dried samples were then converted to graphite by a two-step process using published methods (14). Briefly, the dried samples were oxidized to CO_2 by heating at 900° C for 4 h in the presence of copper oxide. The CO_2 was then cryogenically transferred to a septa-sealed vial under vacuum and reduced to filamentous graphite in the presence of cobalt, titanium hydride, and zinc powder.

Total radiocarbon content in the plasma was quantified by AMS as described previously (14, 19-20). The $^{14}C/^{12}C$ ratios from the graphitized samples obtained by AMS were converted to ng GP-4 per mL of plasma after subtraction of the background carbon contributed from the sample and correction for the specific activity of the ^{14}C-GP-4 dosing material and the carbon content of the sample (12).

Pharmacokinetics. Pharmacokinetic parameters of GP-4 were calculated by non-compartmental analysis using either WinNonlin Professional Software Version 5.2.1 (Pharsight Corp. Mountain View, CA)) or PK Solutions Software (Summit Research Services, Montrose, CO). The half life ($T_{1/2}$) and initial GP-4 concentration ($C_{initial}$) were determined by observations from the concentration versus time data. Area under the curve (AUC) was calculated for intervals 0 to *t* and 0 to ∞ where *t* is the time of the last measurable concentration (24 h) and ∞ is infinity, using the

linear trapezoidal method. Volume of distribution (V_d) was determined based on the AUC determination and reflects the V_d during the elimination phase. The clearance calculation is based on the $AUC_{0-\infty}$ and assumes 100% bioavailability. Additionally, to estimate the absorption and disposition rate constants, a two-compartment open model was fit to the plasma concentration-time profile. Estimated parameters include the absorption rate of GP-4 to the peripheral compartment from the central compartment (K_{12}), the transfer rate of GP-4 from the peripheral compartment to the central compartment (k_{21}), and the elimination rate constant (k_{10}).

RESULTS

Dose Proportionality Study. PK parameters of GP-4 were evaluated over a 10-fold dose range that was designed to capture the potential therapeutic doses. Mean plasma concentrations of GP-4 following single intravenous bolus administrations of 3, 10, or 30 mg/kg of GP-4 to male rats are summarised in Table 1 and illustrated in Figure 1. Calculated mean pharmacokinetic parameters are presented in Table 2.

Following intravenous administration of GP-4, plasma samples were collected at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 hrs. Maximum observed plasma concentrations (C_{max}) of GP-4 were attained at the first sampling time-point (i.e. 5 minutes post-dose) with mean values ranging from 1430 to 13600 ng/mL across the 3 different doses. The mean apparent terminal half-life ranged from 4.4 to 7.9 hrs. Across the 3 doses studied, total clearance of GP-4 from plasma (CL) was 42.1 to 68.0 mL/min/kg and the apparent volume of distribution (V_d) was 26600 to 30400 mL/kg suggesting rapid and extensive distribution beyond the plasma compartment.

The mean C_{max} and AUC_{0-t} values of GP-4 and the relative increase in parameter values between adjacent and overall doses are summarized in Table 3. Following an increase in dose from 3 to 10 mg/kg (3.33-fold), the increases in mean C_{max} (5.11-fold) and AUC_{0-t} (4.83-fold) values were slightly greater than expected for a linear dose-response. However, following an increase in dose from 10 to 30 mg/kg (3 fold), the increase in mean C_{max} (1.86-fold) was slightly lower than dose

proportional, although the increase in AUC_{0-t} (3.27-fold) was approximately dose proportional. Overall, the for an increase in dose from 3 to 30 mg/kg (10-fold), the increases in mean C_{max} (9.51-fold) and AUC_{0-t} (15.8-fold) values were within a factor of two and considered dose proportional.

Pharmacokinetic Linearity Study: Microdose vs. Pharmacologic Dose. A major goal of this study was to determine the effect of dose on PK parameters for GP-4 in rats prior to conducting a microdose study in humans. Male Sprague-Dawley rats received a single bolus IV administration of ^{14}C-GP-4 at either a microdose (0.01 mg/kg) or a pharmacological dose (3 mg/kg), and plasma was collected at designated time points. AMS was used to quantify the plasma concentration of ^{14}C-GP-4 by quantifying ^{14}C equivalents at each time point. The pharmacokinetics at both dose concentrations were then calculated by non-compartmental analysis and compared.

Mean plasma concentrations of GP-4 (based on total radioactivity) following single intravenous dose administrations of 0.01 or 3.0 mg/kg of ^{14}C-GP-4 to male rats are summarised in Table 4 and illustrated in Figures 2. Calculated mean pharmacokinetic parameters are presented in Table 5.

Maximum observed plasma concentrations (C_{max}) of GP-4 were attained at the first sampling time-point (i.e. 5 minutes post-dose). The mean distribution half-life for both compound concentrations was 0.26 hr, whereas the apparent mean terminal half-life was 5.1 hrs and 13.2 hrs for the 0.01mg/kg dose and the 3 mg/kg dose respectively. Total clearance of ^{14}C-GP-4 from plasma (CL) was 2.4-fold higher in the animals that received the 0.01 mg/kg dose (CL=65.2 mL/min/kg) compared to the 3.0 mg/kg dose (CL=26.4 mL/min/kg). The apparent volume of distribution (V_d) was 28552 mL/kg and 30247 mL/kg in the micro and pharmacologic dosed animals, respectively, suggesting rapid and extensive distribution beyond the plasma compartment. Applying a 2-compartment open model, the absorption rate of GP-4 to the peripheral compartment from the central compartment (k_{12}) was 1.66 hr^{-1} and 2.08 hr^{-1} for the microdose and therapeutic dose, respectively. The k_{21} rate constant (transfer rate of GP-4 from the peripheral compartment to the central compartment) for the microdose and therapeutic dose was 0.5 hr^{-1} and 0.22 hr^{-1},

and the elimination rate constant (k_{10}) was 0.70 hr^{-1} and 0.71 hr^{-1}, respectively (data not shown). The higher k_{12} compared to the k_{21} and k_{10} in both doses reinforces the conclusion that the majority of GP-4 is rapidly eliminated from the plasma and distributed to tissues and/or eliminated at both dose levels.

When dose-normalized to 1.0 mg/kg the mean C_{max} for the 0.01 mg/kg and 3 mg/kg dose were 243 ng/mL and 709 ng/mL, respectively (Figure 2B). This is a 2.9-fold difference across the 300-fold difference dose range. The mean AUC_{0-t} values of ^{14}C-GP-4, when dose-normalized to 1.0 mg/kg, were 244.9 ng.hr/mL for the 0.01 mg/kg dose and 451.7 for the 3.0 mg/kg dose, which equates to a 1.8-fold difference between the two doses. Overall, for an increase in dose from 0.01 to 3.0 mg/kg (300-fold), the increases in mean AUC_{0-t} (1.8-fold) and C_{max} (2.9-fold) values were within a factor of two and three, respectively, and considered dose linear. Linear regression analysis of the GP-4 plasma concentration from the microdose as compared to the pharmacologic dose revealed a linear relationship between the two doses with a coefficient of determination (R^2) value of 0.974 and a correlation coefficient (R) of 0.98 indicating a strong association between the two doses (Figure 3).

DISCUSSION

Microdosing is becoming a widely accepted way for estimating human pharmacokinetics at therapeutic dose levels for preclinical drug candidates and has been endorsed by the U.S. Food and Drug Administration and the European Medicines Agency (21-22). Microdosing studies can provide an early determination of the pharmacokinetic properties of a drug candidate, eliminating compounds with poor PK properties and allowing compounds with favorable PK to proceed for further clinical evaluation. Estimating human PK can greatly reduce the attrition rate of new drugs during the later stages of development saving time and reducing costs. Key to the use of microdose studies is establishing a correlation between the PK at the micro- and pharmacological dose. Although the published data are limited, studies that have compared microdose and therapeutic doses have shown that the microdose data are predictive of the therapeutic dose in more than 80% of

the cases (23-24, 6, 8, 11). One approach for establishing dose linearity is to validate the approach in an animal model prior to initiating a human microdosing study (11, 8).

In the present study the rat was used as a model to determine the PK of the trycyclic GyrB/ParE inhibitor drug candidate, GP-4, at microdose and pharmacological dose levels, as a prelude to a human microdosing study. This is the first reported study to assess the PK parameters of this class of drug-candidate. Results showed that the plasma PK was proportional across 3 proposed therapeutic dose levels, indicating linear kinetics in the pharmacological dose range. Additionally, levels of unchanged GP-4 after a 3 mg/kg IV dose, measured by LC/MS/MS, were similar to that of total ^{14}C-content of ^{14}C-GP-4 at the same dose as measured by AMS suggesting little or no metabolism/breakdown of GP-4 at this dose level *in vivo*.

Taking advantage of the increased sensitivity of AMS as compared to LC/MS/MS, PK parameters from animals administered a microdose (0.01 mg.kg) of GP-4 were quantified by AMS. For a direct comparison, AMS was also used to quantify PK parameters at a 3.0 mg/kg pharmacological dose. Based on the results, the PK in the rat is linear across a 300-fold dose range (R^2=0.97). Both the micro- and pharmacological dosed groups exhibited a multi-compartment PK profile, initially showing a rapid decline in plasma concentration post-dose followed by a slower terminal phase. Both dose groups exhibited a large apparent volume of distribution suggesting rapid and extensive distribution beyond the plasma compartment. For an increase in dose from 0.01 to 3.0 mg/kg (300-fold), the increases in mean $AUC_{0\text{-}t}$ (1.8-fold) and C_{max} (2.9-fold) values were within a factor of two and three, respectively, and considered dose linear. Given the rapid distribution observed, estimation of the C_{max}, which was the concentration measured at the earliest (5 minute) collection time from an IV bolus administration, was expected to be quite variable and the C_{max}-to-dose linearity analysis likely reflects this. Therefore, a more pragmatic measure of dose linearity comes from analysis of the $AUC_{0\text{-}t}$-to-dose linearity analysis. Combining and plotting $AUC_{0\text{-}t}$ exposures from the first study (dose proportionality at 3, 10, 30 mg/kg) and the

second study (0.01 and 3 mg/kg) against the dose (Figure 4) provides further confirmation of pharmacokinetic linearity over 4 orders of magnitude. This observed linearity in the rodent model provides assurance that a microdose study in humans can be predictive of the kinetics at a higher therapeutic dose level.

This study provides further evidence that the PK parameters of many drug classes, when administered at 0.01% of the intended therapeutic dose, are linear functions of the administered dose and thus can be used to estimative the PK parameters at a therapeutic dose.

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Table 1. Mean plasma concentrations (± SEM) of GP-4 following a single IV administration of 3, 10, or 30 mg/kg GP-4 to male SD rats (n=3)

Dose	Plasma Concentration (ng/mL) at Time (hr)							
(mg/kg)	0.083	0.25	0.5	1	2	4	8	24
3	1430 ± 315	254 ± 33.1	154 ± 7.2	95.3 ± 1.9	58.1 ± 11.9	20.6 ± 1.9	9.92 ± 3.4	2.29 ± 0.21
10	7310 ± 762	875 ± 98.1	521 ± 91.7	350 ± 72.7	175 ± 38	81.7 ± 16.8	39.7 ± 8.9	15.4 ± 3.2
30	13600 ± 3227	3270 ± 292	2210 ± 161	1430 ± 163	817 ± 131	479 ± 109	250 ± 37.5	98.3 ± 27.6

Table 2. Pharmacokinetic parameters of GP-4 following a single IV administration of 3, 10, or 30 mg/kg GP-4 to male SD rats

Dose (mg/kg)	t_{max} (hr)	C_{max} (ng/mL)	$t_{½}$ (hr)	AUC_{0-t} (ng.hr/mL)	$AUC_{0-\infty}$ (ng.hr/mL)	CL (mL/min/kg)	V_d (mL/kg)
3	0.083	1430	4.4	722	751	68.0	26600
10	0.083	7310	7.7	3490	3660	45.6	30400
30	0.083	13600	7.9	11400	12500	42.1	29600

Table 3. Dose-Exposure Relationship of GP-4 following single intravenous administration of 3, 10, or 30 mg/kg GP-4 to male SD rats

Dose (mg/kg)	Fold Increase	C_{max} (ng/mL)	Fold Increase	AUC_{0-t} (ng.hr/mL)	Fold Increase
3	-	1430	-	722	-
10	3.33	7310	5.11	3490	4.83
30	3.00	13600	1.86	11400	3.27
Overall	10.0		9.51		15.8
DPF			0.951		1.58

DPF = Dose Proportionality Factor (Fold increase in parameter/Fold increase in dose)

Table 4. Mean plasma concentrations (± SEM) of GP-4 following a single IV administration of 0.01 or 3.0 mg/kg ^{14}C-GP-4 to male SD rats (n=3)

Dose	Plasma Concentration (ng/mL) at Time (hr)							
(mg/kg)	0.083	0.25	0.5	1	2	4	8	24
0.01	2.42 ± 0.14	0.67 ± 0.04	0.46 ± 0.03	0.34 ± 0.02	0.23 ± 0.01	0.14 ± 0.09	0.07 ± 0.03	0.01*
3	2126 ± 875	304 ± 45.6	208 ± 15	142 ± 9.1	243 ± 68.7	33.4 ± 3.8	10.6 ± 9.1	28.1*

*value is from n=1

Table 5. Pharmacokinetic parameters of GP-4 following single intravenous administration of 0.01 or 3.0 mg/kg ^{14}C-GP-4 to male SD rats

Dose (mg/kg)	t_{max} (hr)	C_{max} (ng/mL)	$t_{½}$ (hr)	AUC_{0-t} (ng.hr/mL)	$AUC_{0-\infty}$ (ng.hr/mL)	CL (mL/min/kg)	V_d (mL/kg)
0.01	0.083	2.4	5.1	2.5	2.6	65.2	28552
3.0	0.083	2126	13.2	1356	1892	26.4	30247

FIGURE LEGENDS

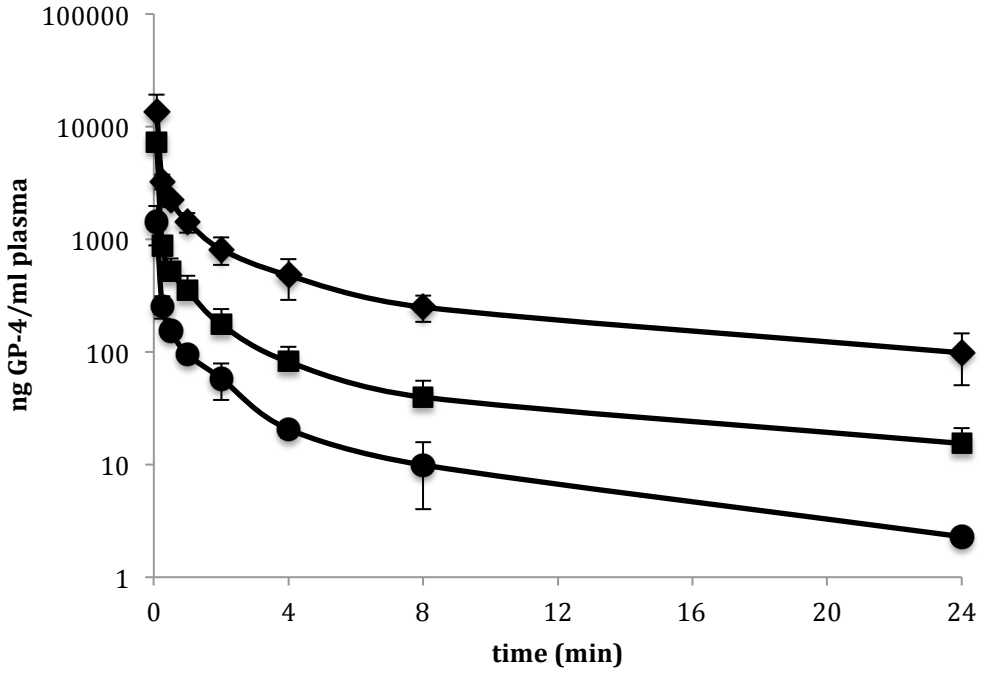
Figure 1 Mean (n=3 +/- SEM) plasma concentration-time profiles of GP-4 following single intravenous administrations of 3 (●), 10 (■), or 30 (◆) mg/kg GP-4 to male SD rats

Figure 2. **A**: Mean plasma concentration-time curve (n=3 ± SEM) of GP-4 following single intravenous administration of 0.01 (●) or 3.0 mg/kg (■) ^{14}C-GP-4 to male SD rats. **B:** Data shown in A normalized to 1 mg/kg dose. *Data point at 24 h is derived from n=1.

Figure 3. Regression analysis of mean microdose plasma concentration of GP-4 vs. mean therapeutic dose plasma concentration of GP-4. Coefficient of determination = 0.974, Correlation coefficient = 0.98

Figure 4. Regression analysis of GP-4 dose vs. AUC_{0-t}. Coefficient of determination = 0.9965

Figure 1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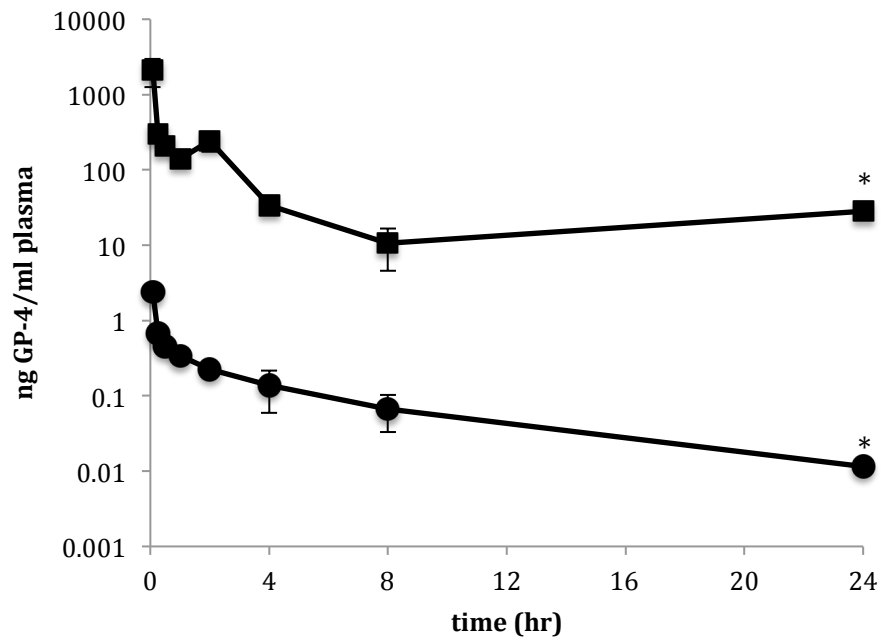
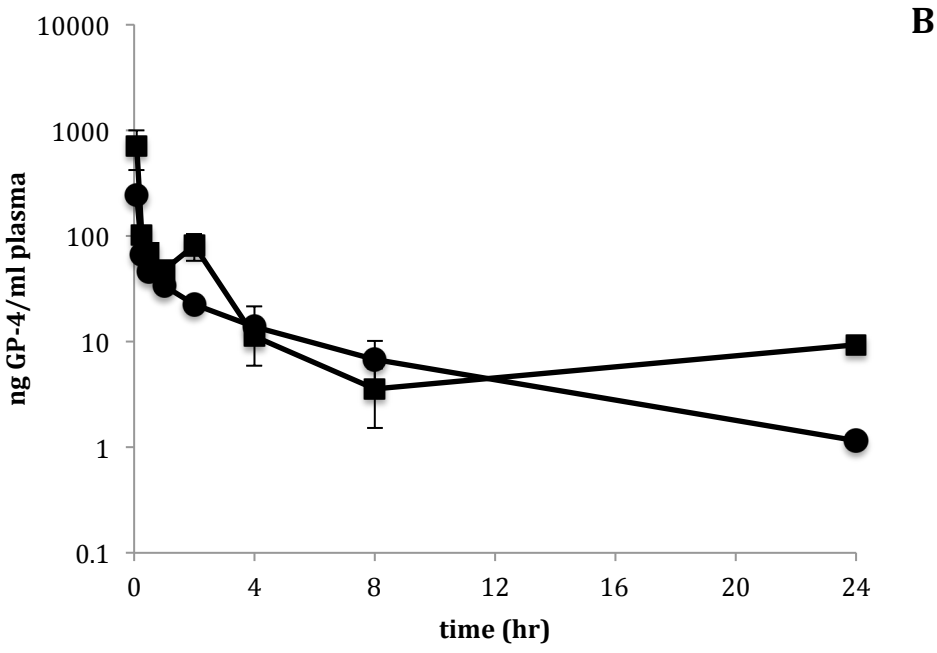
100
10
1
ng GP-4/ml plasma
0
4
8
12
16
20
24
time (min)

Figure 2


A
10000
1000
100
10
1
0.1
0.01
0.001
ng GP-4/ml plasma
*
*
0
4
8
12
16
20
24
time (hr)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10000
1000
100
10
1
0.1
ng GP-4/ml plasma
0
4
8
12
16
20
24
time (hr)

Figure 3

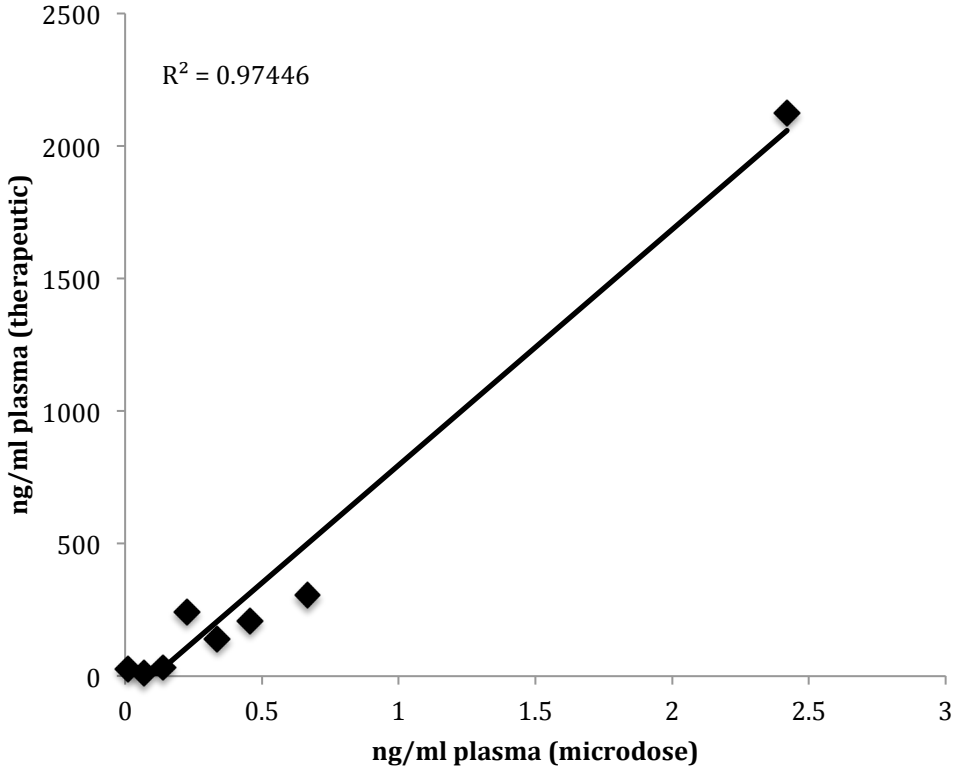
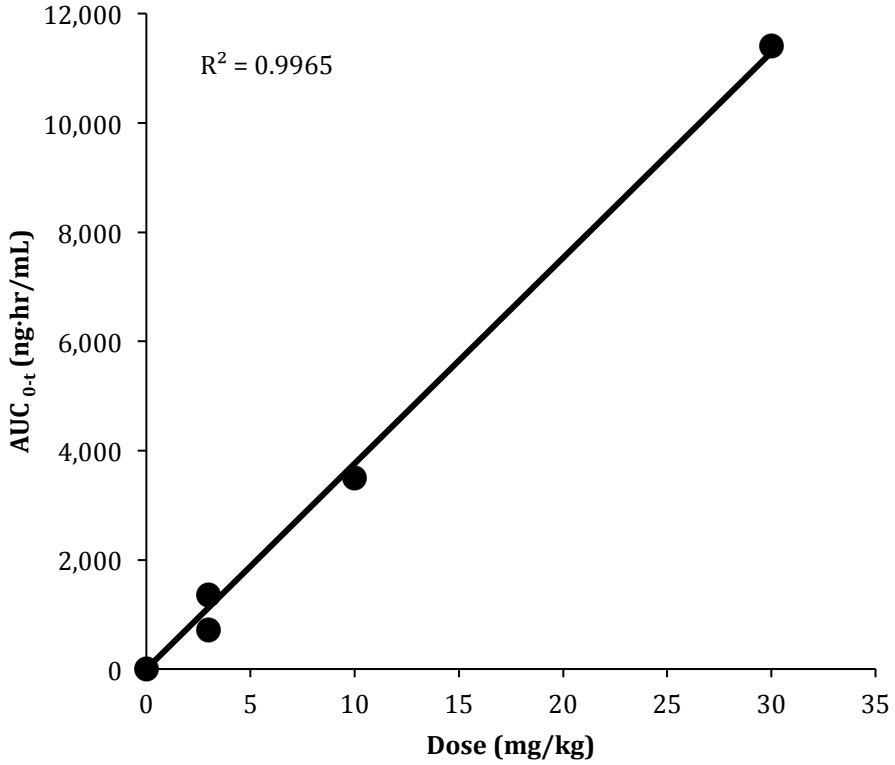

2500
2000
1500
1000
500
0
R^2 = 0.97446
ng/ml plasma (therapeutic)
0
0.5
1
1.5
2
2.5
3
ng/ml plasma (microdose)

Figure 4


12,000
10,000
8,000
6,000
4,000
2,000
0
R^2 = 0.9965
AUC_{0-t} (ng·hr/mL)
0
5
10
15
20
25
30
35
Dose (mg/kg)